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Molecular detection of bacteria from fish lakes in Wasit province

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Fish products are very susceptible to microbial contamination, rendering them perishable thus challenging the customers health. The goal of this study was to determine the molecular identification and bacterial isolates diversity obtained from infected fish skin, gills, and fins obtained from lakes Alzarkan village in Wasit Province of Iraq during the period (October 2021- May 2022). A total of 50 fish were collected and 100 samples of organ lesions were taken. For bacterial identification, the tissuees of the fish were as cut with a sterile knife and blended in the buffered peptone water. Molecular identification and the obtained PCR product were are subjected to using the primers for *Flavobacterium columnare, Edwardsiella ictaluri and Aeromonas salmonicida*. Molecular analyseis revealed bacterial strains high proportion of *Flavobacterium columnare* identification was 20/50 (40%). The higher identification rates of *Flavobacterium columnare* in skin 10/20 (5%), in gills 7/20 (35%) and in fins 3/20 (15%). Accurate identification of the 20 isolated *Flavobacterium columnare* were confirmed by PCR. For identifying certain diseases, molecular approaches provided sensitive, rapid, and precise data without the need for time-consuming traditional techniques. The Edwardsiella ictaluri and Aeromonas salmonicida were not identified. While Flavobacterium columnare termed to be first molecular proof as a farmed fish pathogen in Iraq.
Keywords: Molecular detection, Bacteria, Fish lakes, Wasit province, Iraq.

INTRODUCTION

The name "fish" is frequently used to refer to a class of aquatic, poikilothermic (cold-blooded), gill-breathing vertebrates in the phylum Chordata (Keat-Chuan *et al*., 2017; Nelson, 2006). Fish are classified into two types: cartilaginous fish (such as sharks and stingrays) and bony fish. Fish meat has high nutritional value due to its high protein, vitamin, and fatty acid unsaturated content; it is an important feed stuffs because it has been the cheapest source of animal protein in recent years (Ali *et al*., 2014; Albuquerque *et al*., 2007 and Abdulla, 2003). The development of a specific fish disease is heavily influenced by the conditions of the climate that prevail in the given region, country, or zone. This means that the issues of health differ between fish cultured in the northern European countries, Europe continental, and Mediterranean Sea. In the countries of the Scandinavian countries, one of the most serious health issues is amoebic gill disease (AGD) and copepod invasion. Meanwhile, Infections of *Aeromonas spp*. are the common bacterial diseases of fish in Central Europe, causing motile aeromonas infection (MAI), motile aeromonas septicaemia (MAS), and furunculosis. Flavobacterium spp. caused also common infections (Safińska, 2018). Other organisms besides fish live in water, including many saprophyte bacteria species that inhabit plants and sediments, as well as zooplankton and phyto. Some of them colonize the gills, skin, and digestive tracts of fish like commensals, aiding digestion and improving the immune systems of these animals. Because these microorganisms may endanger fish health, they are classified as conditionally pathogenic. Many studies have been conducted around the world on the interactions among bacteria, fish, and diseases. The high level of interest in these topics demonstrates their importance in fish pathology. The intricate process of disease development depends on the fish immune system as well as the capacity of germs to produce health problems, environmental factors, and disease agent virulence. So, it appears that changes in freshwater ecosystems are crucial for the development of any illness, even emergent ones (Johnson and Paull, 2011; Austin and Austin, 2016; Al-Obaidy and Al-Dabahg, 2011). Pathogenic diseases, which are more common and generically characterized as bacterial infections, are frequently connected with large death and morbidity rates as well as wide-spread harmful repercussions on farmers, consumers, and the environment. It is possible that the sickness is brought on by

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an unbalanced interaction between the host fish (fish), the environment (water), and the chemicals that cause the disease (pathogens). Infections are more likely to infiltrate the fish's body when there are stressful contacts (Mala and Abdullah, 2022). Fish health and production may suffer as a result of feed losses and plant materials used in fish feed being infected with fungus and mycotoxin during crop production or storage. Fish feed is a rich source of protein and other nutrients needed for fish growth and health (Hassan and Hassan, 2020). The production of fish has grown steadily over the world, notably in Iraq, where fish farming dates back to the middle of the nineteenth century (Garabawi *et al*., 2022). Common carp are the fish that are most frequently found in fish farms in Iraq. Silver carp, grass carp, hypophthalmichthys molitrix, and Cyprinus carpio L. A species of Ctenopharyngodon is Ctenopharyngodon idella , Furthermore, these farms may be infected with noninfectious diseases (Garabawi *et al*., 2022). Many pathogenic microorganisms, such as bacteria, viruses, parasites, and protozoa, infect fish farms (Jassim *et al*., 2019). The layers of the skin are crucial barriers for protective fish, allowing them to slide more easily through the water and less energy using by swimming. Additionally, there are some compounds in the mucus which shield the fish from the bacteria in the water and other organisms, making skin disorders in fish particularly dangerous. Surface injuries to the skin also make more difficult fluid balance and should result in circulatory malfunction. Skin ulceration in fish may be caused by physical causes, infectious agents, toxins, immunologic causes, dietary problems, and metabolic changes, precisely, fish move through a sea of diseases. However, any crack in the skin's natural barrier function can allow for the entry of microorganisms that ordinarily inhabit the skin. As a result, fungus, viruses, bacteria, and parasites should all be considered in the differential diagnosis of skin lesions in fish (Law, 2001). The purpose of this syudy was to use molecular identification to identify bacteria from infected fish gills, skin, and fins in Iraq, the Province of Wasit.

MATERIALS AND METHODS

***Fish Samples Collection*:** The sample type slection and collection were carried out in accordance with (Hassan *et al*., 2017). High levels of mortalities with 30 fish daily were observed. An infected carp fish had visible lesions on its skin, fins, and gills. shows figure (1). A total of 50 samples of fish , gills, and fins of infected fish, and roughly 10 g of the contaminated tissue was placed in a used container and homogenized for three minutes in 90 ml of buffered peptone water before being transferred to the lab for DNA extraction. In which bacteria (*Flavobacterium columnare, Edwardsiella ictaluri and Aeromonas salmonicida*) identification with a infected tissue from each fish organs. Two samples from each fish organs lesion were combined to form a single testing sample. From 50 fish that 100 organ lesion samples were collected.

Figure 1. Visible lesions on its skin, fins, and gills

***PCR amplification and DNA extraction*:** The thermal extraction was used to extract DNA samples according to (AL-abedi *et al*., 2020). Following the manufacturer's recommendations. Gel electrophoresis was used to the quality assess of the extracted DNA. Each sample was stained with three microliters (3 µl) of ethidium bromid dye and loaded for 30 minutes at 80 V in a 1% agarose gel in 0.5X (Bio lab) TBE buffer. After being observed with a gel documentation technique, DNA bands were imaged. The Flavobacterium columnare primers used to amplify a 16S rRNA gene the F. columnare - F (5`-CCTGTACCTAATTGGGGAAAAGAGG-3`)and F.columnare-R (5`-GTTGTATACACATCCGAAGTTCCAT-3`), which amplify the (concentration: 10 pmol) 203 bp fragment, Edwardsiella ictaluri the primer E.ictaluri-F (5`-ACTTATCGCCCTCGCAACTC-3`) and E.ictaluri-R (5`-CCTCTGATAAGTGGTTCTCG-3`) which amplify a 178 bp, Aeromonas salmonicida the primer Asal-aopO-F (5`-AGCTCATCCAATGTTCGGTATT-3`) and Asal-aopO-R (5`-AAGTTCATCGTGCTGTTCCA-3`) which amplify a 199 bp (30,31,33). The PCR amplification was performed in the total volume of 25 µl, which included 12.5 µl of the hot start premix (GoTag Green Master Mix) 1 µl of each reverse and forward primer, 2 µl of sample DNA (50-150ng/µl), and 8.5 µl water nuclease-free (Qiagen, Germany). The thermocycler (GeneAmp® PCR System 9700, Singapore, Applied Biosystems) was used to perform the amplification. The PCR apparatus was configured for 30 cycles. as shown in (Table 1).

Table 1. PCR program setting for *Flavobacterium columnare, Edwardsiella ictaluri* and *Aeromonas salmonicida*.

No.	Step	*Flavobacterium columnare*	*Edwardsiella ictaluri*	*Aeromonas salmonicida*	Time	
1	Initial denaturation	95˚C	95˚C	95˚C	2 min	
2	Denaturation	95˚C	95˚C	95˚C	30 sec	
3	Annealing	60˚C	62˚C	62˚C	30 sec	30 cycle
4	Extension	72˚C	72˚C	72˚C	50-60 sec	
5	Final Extension		72°C		5 min	
6	Hold		4°C		10 min	

RESULTS

Fifteen samples were collected and analyzed from carp fish, in the present study, the results of the amplified 16S rRNA gene for molecular identification bacteria were *Flavobacterium columnare*, but not identification other species bacteria *Edwardsiella ictaluri* and *Aeromonas salmonicida*. The percentage of *Flavobacterium columnare* identification was 20/50 (40%). the higher identification rates of *Flavobacterium columnare* in skin 10/20 (5%), in gills 7/20 (35%), in fins 3/20 (15%). Accurate identification of the 20 isolates of *Flavobacterium columnare* were confirmed by PCR (Figures 2).

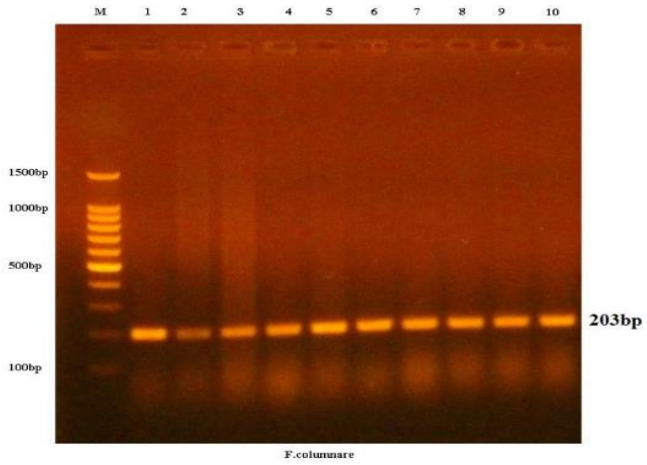

Figure 2. The PCR product analysis of pathogenic *F. columnare* is shown on an agarose gel electrophoresis image. Marker ladder Lane M (1000 bp), lanes 1-10: *F. columnare* isolate 16S rRNA gene (203 bp).

DISCUSSION

Fish and the products of fishery could be a major source of many important species of food poising bacteria, acting as vehicles for them (Haifaa *et al*., 2014). In this study molecular identification 20 isolates species of *Flavobacterium columnare* was 20/50 (40%). The higher identification rates of *Flavobacterium columnare* in skin 10/20 (5%) , in gills 7/20 (35%), in fins 3/20 (15%) were found. Flavobacterial diseases pose the significant threat to global farming of the fish and fish stocks. *Flavobacterium spp*. numerous discovered in association to fish and have been confirmed and/or implicated as pathogens (33 Columnaris disease is brought on by the fatal fish infection *Flavobacterium columnare*, which affects populations of both wild and cultivated fish worldwide (LaFrentz *et al*., 2022; Faisal *et al*., 2017). were found mortality rates are common in aquaculture, resulting in economic significant losses, because of fish mortality, decreased the activity of feeding during epizootics, and higher treatment costs (Peterman and Posadas, 2019). Herbert Spencer Davis first discovered Columnaris illness in 1917, suggesting the name Bacillus columnaris as a result of the bacteria's propensity to form aggregates that resemble columns upon material scraped from affected fish wounds (LaFrentz *et al*., 2022). Davis completed a thorough analysis of the illness and agent despite his inability to grow the bacteria, which included data on the prevalence and causes of columnaris disease, pathogenesis, the mode of infection, and treatments and control measures (LaFrentz *et al*., 2022). Another barrier to bacterial isolation is the specific medium lack for culturing F. columnare (Decostere *et al*., 1998) discussed how difficult it is to obtain pure colonies. As a result, for *F. columnare* confirmation, species-specific chain reaction polymerase (PCR) based on 16S rDNA is necessary (Bader *et al*., 2003 and Darwish *et al*., 2004). In our study the percentage identification of *Flavobacterium columnare* was 20/50 (40%) higher than study in Brazil (Pilarski *et al*., 2008) reported the different species bacteria include *Proteus spp*. (2) isolates, *Shigella spp*. (5) isolates, *Providencia spp*. (6) isolates, *Citrobacter spp*. (8) isolates, *Acinetobacter spp*. (10) isolates and E. coli (17) isolates.

According to (Garabawi *et al*., 2022), it is possible that the higher rate of infection in fish harvested from cages compared to fish harvested from ponds is due to the most dangerous bacteria linked to skin ulceration in both cages and ponds in all three locations in Wasit province. These bacteria are linked to the type of fish feed given to the fish, location of sampling sites, nearby human activities, and water quality at fish rearing areas. In the District of Taqtaq in Erbil Province, the Kurdistan Region of Iraq, the fish were examined for bacteria this research the fortune revealed (13) bacteria species

(*Methylobacterium spp, Streptpcoccus equi zooepidemicus, Sphingobacterium thalpophilum, B. stabilis, Burkholderia cepacia, B. vietnamiensis, B. multivorance,* and *Rhizobium radiobacter*) are considered in Iraq the first record, and the five bacteria species (Pseudomonas aeruginosa, *Staphylococcus lentus, Serratia fonticola, Klebsiella oxytoca,* and *Streptococcus thoraltensis*) are recorded previously in the Kurdistan Region (Mala and Abdullah, 2022). In epidemiological studies, molecular characterization has been used to safely and efficiently identify different pathogen genotypes. Furthermore, these techniques are more efficient than other methods. Recognizing prevalence of the specific genotype and its patterns in an infection can lead to the development of more effective ichtiopathology control methods. The molecular results in our study were consistent with the results of a study in Brazil (Sebastião *et al*., 2010) which suggested molecular techniques, with PCR is the most effective method for identifying *columnaris.* The most practical method was the use of particular primers for conserved areas of bacterial DNA because there was a dearth of information on the genome sequence of *F. columnare* from hosts in aquatic and tropical climates.

***Conclusion*:** It is notable that *Flavobacterium columnare* was molecularly identified in Wasit Province because this is the first time this dangerous fish pathogen has been reported in this nation. It has been shown that the use of PCR for bacterial identification is a quicker and more efficient way than traditional biochemical methods, which are time-consuming and frequently inconclusive, due to the high mortality rate of carp fish. Nonetheless, further research is in progress to improve the ability to extract Flavobacterium and other microbes in tropical fish farms.

***Authors contributions statement*:** Semaa F.H. AL-Abedi: Run the analyses and wrote the article; Conceived the idea and designed the study. Also collect data and run the analyses and writing.

***Conflict of interest*:** The authors declare that there is no conflict of interest.

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***Ethical statement*:** Ethical approve OR data collection permit UM.VET.2021.072.

***Availability of data and material*:** We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere Code Availability: Not applicable

REFERENCES

Abdulla, R.K. 2003. Nutritional value of fish. Arabian Scientific Res. J. 1:16 - 20.

AL-abedi, H.F.H., A.A.F. AL-Attraqchi and B.Y.Khudaier. 2020. Anti-pathogenic Candida Spp activity determination via Lactobacillus Spp. isolation and identification using conventional and molecular methods. Basrah J. Vet. Res. 19:130-148.

Albuquerque, W.F., A. Macrae, O.V. Sousa, G.H.F. Vierira and R.H.F. Vieira. 2007. Multiple drug resistant Staphylococcus aureus strain isolated from a fish market and from fish handlers. Brazilian Journal of Microbiology 38:131-134.

Al-Obaidy, N.K. and S.Y. Al-Dabahg. 2011. Isolated and identification of pathogenic bacteria from intestine of Cyprinus caprio fish from Tigris river in Mosul city. Journal of Education and Science 3: (in Arabic).

Austin, B. and D.A. Austin. 2016. Bacterial fish pathogens. Disease of farmed and wild fish, 6th ed. Springer International Publishing Switzerland; 2016.

Bader, J.A., C.A. Shoemaker and P.H. Klesius. 2003. Rapid detection of columnaris disease in channel catfish (*Ictalurus punctatus*) with a new species-specific 16-S rRNA gene-based PCR primer for Flavobacterium columnare. Journal Microbiol. Methods 52:209-220

Darwish, A.M., A.A. Ismaiel, J.C. Newton and J. Tang. 2004. Identification of Flavobacterium columnare by a species-specific polymerase chain reaction and renaming of ATCC43622 strain to Flavobacterium johnsoniae. Molecular and Cellular Probe 18:421– 427. DOI: 10.1016/j.mcp.2004.07.002.

Decostere, A., F. Haesebrouck and L.A. Devriese. 1998. Characterization of four Flavobacterium columnare (*Flexibacter columnaris*) strains isolated from tropical fish. Vet. Microbiol 62:35-45.

Faisal, M., A. Diamanka. T.P. Loch, B.R. LaFrentz, A.D. Winters, J.C. García and B.S. Toguebaye. 2017. Isolation and characterization of Flavobacterium columnare strains infecting fishes inhabiting the Laurentian Great Lakes basin. Journal of Fish Diseases 40:637-648.

Garabawi, A.J., J.K. Al-Faragi and K.Y. Zakair. 2022. Detection of the most important pathogenic bacteria affect external organs of Cyprinus carpio in Wasit province. Iraqi J. Agric. Sci. 53:1115-1122.

Haifaa H. A. 2014. Isolation and identification of staphylococcus bacteria from fish of fresh water and its antibiotic sensitivity in Mosul city. Basrah J. Vet. Res. 1:33-42.

Hassan, A.S., F.F. Hassan. 2020. Isolation and identification of fungi from fish feedstuff of cyprinus carpio and

detection of aflatoxin b1 and ochratoxin a using ELISA technique. Annals of Tropical Medicine and Public Health, 23, 232-448.

Hassan, M.A., E.A. Noureldin, M.A. Mahmoud, N.A. Fita. 2017. Molecular identification and epizootiology of *Aeromonas veronii* infection among farmed *Oreochromis niloticus* in Eastern Province, KSA. The Egyptian Journal of Aquatic Research 43:161-7.

Jassim, A.A.R., D.B. Abdulhameed and N.R. Al Shammari. 2019. Bacterial Fish Diseases in some Semi-close Aquaculture Systems in Basrah Province, Iraq. Basrah Journal of Agricultural Sciences Basrah 32: 75-84.

Johnson, P.J. and S.H. Paull. 2011. The ecology and emergence of diseases in fresh waters. Freshwater Biolgy 56:638-657.

Keat-Chuan, Ng.C, O.P. Aun-Chuan, W.L. Wong, G.A. Khoo. 2017. Review of Fish Taxonomy Conventions and Species Identification Techniques. Journal of Survey in Fisheries Sciences 4:54-93:

LaFrentz, B.R., S. Králová, C.R. Burbick, T.L. Alexander, T.P. Loch, M.R. Liles and K.R. Snekvik. 2022. The fish pathogen *Flavobacterium columnare* represents four distinct species: *Flavobacterium columnare*, *Flavobacterium covae* sp. nov., *Flavobacterium davisii* sp. nov. and *Flavobacterium oreochromis* sp. nov., and emended description of Flavobacterium columnare. Systematic and Applied Microbiology 45:

Law, M. 2001. Differential diagnosis of ulcerative lesions in fish. Environmental Health Perspectives 109:681-686.

Mala, H.M. and S.M.A. Abdullah. 2022. Isolation and identification of some bacterial species from common carp (*Cyprinus carpio* Linnaeus, 1758) in Taqtaq district in Erbil Province, Kurdistan Region, Iraq. ZANCO Journal of Pure and Applied Sciences 34:131-140.

Nelson, J.S. 2006. Fishes of the World, 4th Edition. New Jersey: John Wiley & Sons Inc. 601.

Peterman, M.A. and B.C. Posadas 2019. Direct economic impact of fish diseases on the East Mississippi catfish industry. North American Journal of Aquaculture 81:222-229.

Pilarski, F., A.J. Rossini and P.S. Ceccarelli 2008. Isolation and characterization of *Flavobacterium columnare* (Bernardet *et al*. 2002) from four tropical fish species in Brazil. Brazilian Journal of Biology 68:409-414.

Safińska, A.P. 2018. Contemporary Threats of Bacterial Infections in Freshwater Fish. Journal of Veterinary Research 62:261-267.

Sebastião, F.A., F. Pilarski, and M.V.F. Lemos 2010. Isolation and molecular characterization of *Flavobacterium columnare* strains from fish in Brazil**.** Journal of Bacteriology Research 2:22-29.